PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(54) Title: CAPILLARY ARRAY CONFOCAL FLUORESCENCE SCANNER AND METHOD
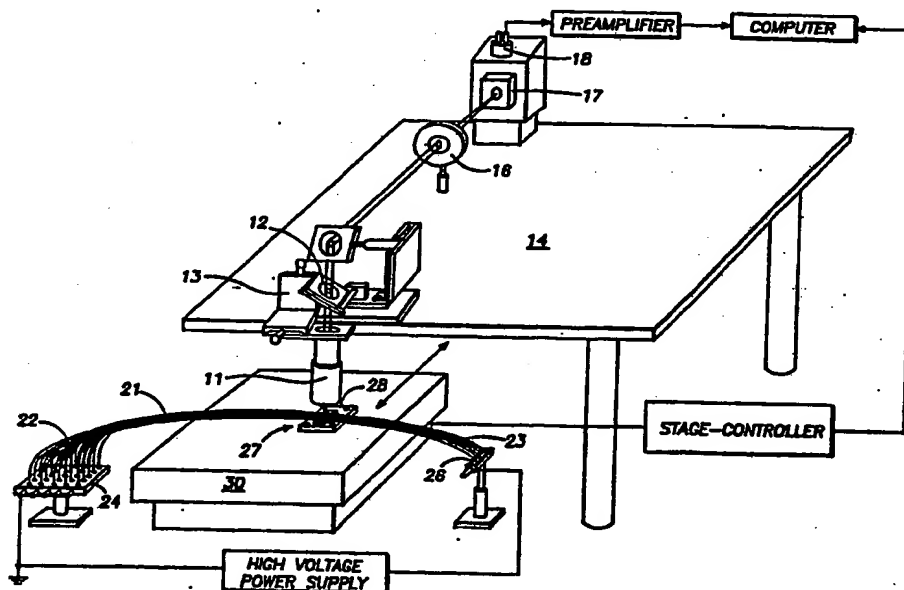
(57) Abstract

A laser-excited capillary array scanner including a plurality of capillaries (21) having a parallel, side-by-side, coplanar relati nship and a laser-excited confocal fluorescence detect r (18) f r detecting fluorescence from a selected interior volumes of each of said capillaries (21) sequentially and repetitively during electrophoresis or other separation method. The invention also relates to a method f analyzing a plurality of capillaries (21), with a single scanner (30), by scanning a plurality of capillary passages in side-by-side relati nship, and periodically and repetitively detecting fl rescence from each capillary passage during electrophoresis or any other separation procedure.

-1-

CAPILLARY ARRAY CONFOCAL FLUORESCENCE
SCANNER AND METHOD

Brief Description of the Invention

This invention relates generally to a capillary array
5 scanner and method, and more particularly, to a capillary
array confocal fluorescence scanner and method for detecting
electrophoretic, chromatographic or other separations
performed on arrays of capillaries.

Capillary electrophoresis (CE) has found widespread
10 application in analytical and biomedical research, and the
scope and sophistication of CE is still rapidly advancing[1-5].
Gel-filled capillaries have been employed for the rapid
separation and analysis of synthetic polynucleotides[6], DNA
sequencing fragments[7-11] and DNA restriction fragments[12,13].
15 Open-tube capillary electrophoresis has attained subattomole
detection levels in amino acid separations[14], and proven its
utility for the separation of proteins, viruses and
bacteria[15]. Separation of the optical isomers of dansyl
amino acids has also been successfully demonstrated[16].
20 Micellar electrokinetic capillary chromatography,
isoelectric focusing, and on-column derivation can all be
performed in capillary columns, demonstrating the utility of
capillaries as an analytical and micropreparative tool[4,5].

The advantages of CE arise intrinsically from the use
25 of a small inside diameter (20-200 μm) capillary. High
electric fields can be applied along small diameter fused-
silica capillaries without a significant increase in the
temperatur of the separation medium or column. Since the
electrophoretic velocity f the charged species is

proportional to th applied field, CE can achieve rapid,
high-resolution separation. The reduced Joule-heating in CE
is a result of the very low current passing through the
capillary, the large surface-to-volume ratio of the
5 capillary channel, the use of thin capillary walls (~50-
150μm), and the high thermal conductivity of the wall
material [1].

Although CE provides rapid analysis, thus far the total
throughput is not high because only one capillary can be
10 analyzed at a time. Developing a method to increase the
throughput of CE is a challenging and important task. One
possible approach is to employ a much higher electric field
which would provide faster separations. Higher electric
fields, however, often introduce overheating of the columns
15 and column failure.

Another way to increase the throughput is to run a
large number of capillary separations in parallel. This
approach uses an array of capillaries and is therefore
called capillary array electrophoresis (CAE). CAE is
20 potentially advantageous because the individual capillaries
can be independently manipulated at the inlet, thereby
facilitating rapid, parallel loading of multiple samples.
In our approach, the capillaries are combined into a ribbon
at the outlet for ease of parallel, on-column detection. In
25 this way, a two order-of-magnitude increase in CE throughput
should be achieved because hundreds of capillaries can be
easily bundled for detection.

An important problem confronting capillary array
electrophoresis is detection. Since small amounts of sample
30 are injected in a capillary, a high-sensitivity detection
system is indispensable. Laser-excited fluorescence has
proven to be a sensitive detection method in capillary
electrophoresis and in DNA sequencing[7-11, 14, 17-21]. In most
laser-excited fluorescence detection schemes, the incident
35 laser beam and the emitted fluorescence are perpendicular to
each other. It is difficult to configure a system to detect
an array f capillaries using this geometry. We have
recently introduced a laser-excited, confocal-flu rescence

gel scanner which provides nhanced detection of fluorescently labeled DNA in slab gels[22-26]. This detection system us s an epi-illumination format where the laser is focused on th sample by a microscope objective and the

5 emitted fluorescence is gathered by the same objective using a 180° geometry followed by confocal detection. This geometry is ideal for on-column detection of capillaries. Using confocal excitation and detection, the depth of a field of the optical system is sufficiently small that only

10 the interior of the capillary is probed. Background scattering, stray fluorescence and reflections from capillary wall are rejected by spatial and spectroscopic filters. Also, the high numerical aperture of the microscope objective gathers the fluorescence very

15 efficien y and presents a high quality image to the pinhole spatial lter. The utility of fluorescence microscope detecti n for CE has been recognized in previous studies using static optical systems to detect single capillaries[27,28].

20 We show here that the ideal way to use a confocal fluorescence detector to detect an array of capillaries is to scan the capillary array past the detector. This format has several advantages that enhance the signal-to-noise ratio: (1) the entire cross-section of the electrophoresis

25 band is sampled as it passes down the capillary and through the detection region; (2) problems due to photobleaching of the band that limit the sensitivity are minimized because the entire band is sampled and the optical system is constantly moving across the band being sampled; and (3) the

30 cylindrical lens effect of the capillary walls permits extended detection of the separation channel that enhances the signal-to-noise ratio. These advantages mean that confocal scanning is a uniquely powerful way to perform high-sensitivity detections of separations on an array of

35 capillary columns.

Objects and Summary of the Invention

It is an object of this invention to provide a method and apparatus for increasing throughput in capillary separations.

It is another object of this invention to provide a method and apparatus for scanning an array of capillaries to detect separations of substances in the capillaries.

It is a further object of this invention to provide a high sensitivity fluorescence detection system for analyzing the interior of a number of parallel capillaries.

It is a further object of this invention to provide an apparatus and method for analyzing a number of separations in an array of capillaries which can be independently manipulated at their inlet to facilitate parallel loading and which are combined in a ribbon array for detection of the separations by a confocal scanner.

It is still a further object of this invention to provide a laser-excited confocal fluorescence detection system and method for analyzing during electrophoresis the gel in the interior of each capillary of a capillary ribbon comprising a plurality of capillaries disposed in side -by-side relationship.

It is still a further object of this invention to provide a laser-excited fluorescence capillary scanner for scanning an array of capillaries during CE.

It is a further object of this invention to provide a laser-excited fluorescence scanner which detects separations across the capillary channel to sample the entire separation band.

It is a further object of this invention to provide an apparatus and method for analyzing DNA sequencing in an array of capillaries which can be independently manipulated at their inlet to facilitate parallel loading and which are combined in a ribbon array for detection of the separations by a confocal scanner.

It is still a further object of this invention to provide a las r-excited confocal fluorescence detection system and method for analyzing during gel electr phoresis

-5-

DNA fragm nts in ach capillary of a capillary ribbon comprising a plurality of gel-filled capillaries disposed in side-by-side r lationship.

It is a further object of this invention to provide a
5   capillary scanner which avoids photobleaching of the sample in the capillary.

It is still a further object of this invention to provide a capillary array scanner in which the capillaries are shaped to provide a continuous sampling of the capillary
10   volume.

These and other objects of the invention are achieved by a laser-excited capillary array scanner including a plurality of capillaries having a parallel, side-by-side, coplanar relationship and a laser-excited confocal
15   fluorescence detector for detecting fluorescence from a selected interior volumes of each of said capillaries sequentially and repetitively during electrophoresis or other separation method. The invention also relates to a method of analyzing a plurality of capillaries, with a
20   single scanner, by scanning a plurality of capillary passages in side-by-side relationship, and periodically and repetitively detecting fluorescence from each capillary passage during electrophoresis or any other separation procedure.

25   The foregoing and other objects of the invention will be more clearly understood from the following description when read in connection with the accompanying drawings, wherein:

Figure 1 is a schematic diagram of a confocal-
30   fluorescence capillary array scanner in accordance with one embodiment of the invention;

Figure 2 is a view of a holder for supporting a region of the capillaries in side-by-side relationship;

Figure 3 is an enlarged view of the focal zone;

35   Figures 4A and 4B illustrate how the excitation beam is focused to a volume in th interior of a cylindrical capillary;

Figure 5 is an image obtained by scanning a four-capillary array during a DNA separation;

Figure 6 is an el ctropherogram of the DNA separation of Figure 5;

5   Figure 7 is an expanded view of the indicated regions of the electropherograms of Figure 6;

Figure 8 is an image obtained by scanning a twenty-four capillary array; and

Figure 9 is a schematic diagram of a four-color
10   confocal-fluorescence capillary scanner.

In accordance with this invention, the throughput in capillary electrophoresis is increased by employing a large number of capillaries in parallel. The most important problem confronting capillary array electrophoresis is
15   detection. In copending patent application Serial No. 07/531,900 filed June 1, 1990, and incorporated herein by reference, there is described a laser-excited confocal fluorescence gel scanner which provides enhanced detection of fluorescently labelled DNA in slab gels. This detection
20   system uses an epi-illumination format where the laser is focused on the sample by a microscope objective and the emitted fluorescence is gathered by the same objective using a 180° retro-optical geometry followed by confocal detection.

25   Sensitive detection of fluorescently-labeled analytes separated in small diameter capillaries is a difficult task. Because the capillaries have a 100 μm I.D. or less, a small focal volume is needed. The detection system must reject potentially strong Rayleigh scattering, fluorescence, and
30   reflections from the capillary walls. Using confocal excitation and detection, the depth of field of the optical system is sufficiently small that only the interior of the 100 μm I.D. capillary is probed. The lateral resolution which is dictated by the scan stage and the laser beam
35   diameter can be as small as a few microns. Background scattering and reflections from the capillary wall are rejected by the spatial and spectroscopic filters in front f the photodetector.

A confocal fluoresc nce detection system for us with
capillary arrays is shown in Figur 1. An argon ion laser
(Model 2020, Sp ctra-Physics, Mountain View, California),
n t shown, is used as the excitation source. The laser beam
5   is expanded to 5 mm diameter, collimated, and then directed
through a 32X, N.A. 0.4 infinite conjugate objective 11 (LD
Plan-Achromat 440850, Carl Zeiss, West Germany) by a long-
pass dichroic beamsplitter 12 (480DM, Omega Optical,
Brattleboro, VT). The dichroic beam splitter 12 reflects
10 the excitation laser beam into the objective 11 but
transmits fluorescent light collected by the objective which
is Stokes shifted to longer wavelengths. The objective
focuses the exciting laser on the sample and gathers the
fluorescence with very high collection efficiency. The use
15 of an infinite conjugate objective permits vertical
adjustment of the probe volume by translating the objective
with the mount 13 secured to the base 14 with no significant
perturbation of the optical alignment. The focused 1 mW,
488 nm wavelength beam is focused to a 10 μm beam diameter
20 and a 25μm confocal beam parameter. The fluorescence
emission is passed back through the long-pass dichroic beam
splitter 12 mounted on the base 14 to reduce laser
interference and to separate the excitation and detection
paths. The fluorescence is then focused by a 75mm focal
25 length lens 16 mounted on the base 14 onto a 400μm pinhole
which serves as the confocal spatial filter. The light
passing through the pinhole is filtered by a 488nm rejection
band filter (488 RB filter, Omega Optical, Brattleboro, VT),
a long-pass cutoff filter (Schott GG-495, Esco, Oakridge,
30 NJ), a bandpass fluorescence filter (530 DF60, Omega
Optical, Brattleboro, VT), all mounted within the housing
17, followed by detection with a cooled photomultiplier tube
18 (RCA 31034A, Burle Industries, Lancaster, PA). The
spatial filter, the optical filters and photomultiplier tube
35 are mounted on base 14. The output of the phototube is
amplified and filtered with a low-noise amplifier (SR560,
Stanford Research Systems, Sunnyvale, CA), digitized with a
12 bit analog-to-digital board (DASH-16F, Metra-Byte,

Taunton, MA) and stored in an IBM PS/2 microcomputer. The electronic filter used for the phototube output was a first-order, active, low-pass filter (DC to 400 Hz) with a 12 dB/octave rolloff.

5   The capillary array comprises a plurality of capillaries 21 having their ends 22,23 extending into wells 24, 26 between which a high voltage is applied for electrophoresis. The ends 22 may be separated for individual manipulation and loading. A portion 27 of the
10 capillaries is maintained in side-by-side parallel coplanar relationship by a holder 28, Figure 2. The holder 28 includes a window through which the beam can be focused on the interior volume of the capillaries. Figure 3 shows the beam 29 focused in an interior volume of a capillary 21a.

15   For several reasons, scanning the beam and detection system across the capillary is *better* than just probing in the center of the capillary. First, if the probe laser is fixed at the center of the capillary, the sample stream will be rapidly photo-bleached by the laser. Scanning the beam
20 laterally across the capillary interior is much better than sitting in one spot because all of the band is probed (laterally) and photo-bleaching is reduced. Also, the off-axis probing is advantageous because, as shown in Figures 4A and 4B, the cylindrical lens effect actually brings the beam
25 waist back into the capillary gel so the detection system probes the gel for a longer period of time during the scan than would have been nominally predicted from the capillary diameter and scan rate.

The holder 28 is mounted on a translation stage 30
30 (Model 4000, Design Components, Franklin, MA). The stage is programmed to continuously scan the capillary array back and forth at 20 mm/sec in a direction perpendicular to the electrophoresis direction. The image acquired in this way has two dimensions. One is a spatial dimension representing
35 the physical image of the capillaries. The other is a temporal dimension proportional to the elapsed time. During a particular swe p, fluorescence data from the photodet ctor is sampled at 2000 Hz so the nominal image resolution is

10μm/pixel; thus, 10 pixels represent the interior 100μm
width of any given capillary. The electronic low-pass
filter cutoff was set at 300 Hz to provide high frequency
noise rejection while still passing the spatial frequencies
required to define the 100 μm I.D. of the capillaries. An
image of the migrating bands is built up as a function of
time by accumulating periodic one-second sweeps of the
illuminated region of the capillaries. The transit time of
the migrating DNA past the probe region, under the
conditions employed here, ranges from approximately 10
seconds for the low molecular weight fragments (40-50 mers)
to 14 seconds for the higher molecular weight fragments
(380-390 mers). With one-second repeat cycles, this gives
10-14 samples of each band. The computer processes the data
and displays the acquired image in real time.   Image
processing can be performed with the NIH program, Image
1.29, and commercial image processing package, Canvas™, to
provide an image, Figure 5. The image data can be reduced
to a one-dimensional line plot or electropherogram by
averaging the pixels across the width of each lane using
Image 1.29, Figure 6, and sections can be expanded as shown
in Figure 7.

        In one example, zero-crosslinked polyacrylamide gel-
filled capillaries were prepared using a modified method of
the procedure described by Cohen, et al.[6,7]. A 3 mm wide
detection window was produced in each 100 μm I.D. 200 μm
O.D. fused-silica capillaries (Polymicro Technologies,
Phoenix, AZ) by burning off the polyimide coating with a hot
wire. The window was burned ~ 25 cm from the inlet side of
the 40 cm long capillary. The inner wall of the capillaries
was then treated overnight with a bifunctional reagent, γ-
methacryloxypropyltrimethoxy-silane to prepare the walls for
acrylamide adhesion[6]. Freshly-made acrylamide gel solution
(9%T, 0%C) in a 1X TBE buffer (tris-boric acid-EDTA) with 7M
urea was filtered with an 0.2μm syringe filter and degassed
under   vacuum   for   about   ne hour.   10%   TEMED
(tetraethylmethylenediamine)   and   10%   APS   (ammonium
persulfate) solution were added to the gel solution at a

-10-

final concentration of approximately 0.03%. The solution
was immediately vacuum siphoned into the capillaries and
then allowed to p lymerize overnight in a cold room. Prior
to use, both ends of the column wer trimmed by ab ut 1 cm

5   and then pre-electrophoresed for 30 to 60 minutes at 7 kV.

The capillary array was sandwiched in the capillary
holder 28 that is mounted onto the translation stage 30.
The capillary holder 28, Figure 2, serves the dual purpose
of (1) uniformly constraining each capillary in the array to

10 an identical height above the top of the translation stage,
and (2) exposing a small window through which the confocal
zone probed the capillary interior. Constraining the
capillaries to substantially the same plane is necessary for
achieving uniform detection sensitivity from each capillary

15 because the depth of focus of the microscope objective is
only ~ 25-50 μm.

The DNA samples for which the data is shown in Figures
5-7 was prepared as follows: chain-terminated M13mp18 DNA
fragments were generated using a Sequenase 2.0 sequencing

20 kit (U.S. Biochemical Corp., Cleveland, OH) and fluorescein-
tagged primer "FAM" (Applied Biosystems, Foster City, CA).
The detailed procedure has been published elsewhere[25].
Briefly, about one pmol of the primer and single-stranded
M13mp18DNA were heated to 65°C for three minutes and then

25 allowed to cool (annealing reaction). Meanwhile, the
sequencing extension mixture was added into a centrifuge
tube followed by addition of the dideoxy termination
mixture. When the temperature of the annealing reaction
mixture drops below 30°C, a combination of the labeling

30 mixture and diluted enzyme (Sequenase 2.0™) were added, and
the mixture was incubated for five minutes at room
temperature. This mixture was then transferred to the tube
having the termination mixture and allowed to incubate for
another five minutes at 37 °C. Instead of adding stop

35 solution, ethanol precipitation was immediately used to
terminate the reacti n and recover the DNA sequencing
sample. The high concentration of conductive ions present
in the DNA sequencing sample after the termination step

would reduce th amount f DNA that can be loaded into each capillary by electrokinetic inj ction. To counteract this effect, thanol precipitation was performed on all DNA samples followed by resusp nsion in $6\mu l$ of 80% (v/v) forma-
5   mide to give a concentration about ten-fold higher than that used in slab gels. The sample was heated at 90°C for three minutes to ensure denaturation and then placed on ice until sample injection.

The flexibility of the capillary columns allows
10   coupling of the individual capillaries of the array to individual sample wells. In the foregoing example, since only one DNA sequencing sample was run, the sample was placed in a single $500\mu l$ centrifuge tube for electrokinetic injection into the capillaries. The same electric field
15   strength (200 volt/cm) used during separation was also applied during sample injection. The typical injection time was ten seconds. After injection, the inlets of the capillaries were removed from the centrifuge tube and placed into a buffer reservoir or well 24 filled with fresh running
20   buffer. The 9% T gels are sufficiently stable that four to five sequencing runs could be run on each capillary.

Figure 5 presents an image obtained from on-line confocal scanning of a four-capillary array during electrophoresis of a mixture of DNA sequencing fragments.
25   The horizontal direction is the physical dimension representing the geometric arrangement of the array while the vertical direction is temporal, representing the passage of fluorescent DNA fragments through the detection window. For lane-to-lane comparison, identical samples of "G" base
30   DNA fragments were simultaneously, electrokinetically injected into each capillary. The overall elapsed data acquisition time is ~80 minutes after passage of the primer. An expanded region of the image is included in Figure 5. The bands in all four lanes are well resolved and the
35   resolution extends throughout the sequencing run with sufficient signal-to-noise to detect bands more than 500 bas s beyond th primer. From Figure 5, ne can cl arly see

that the cylindrical capillaries do *not* significantly distort the image.

   Figures 6 and 7 present line plots of the DNA signal integrated across the width f each capillary. A signal-to-noise ratio of approximately 20 is observed out to base 385 (~65 minutes) and bands are detected out to base 500 with the present experimental conditions. The number of theoretical plates is >1.9 x 10^6 (at base 385) over a 24 cm effective column length.

   A comparison was made between the signal-to-noise ratio obtained in the scanning mode and the case where the system is focused in the center of a single stationary capillary. The latter approach is analogous to traditional on-column detection from a single capillary. The sensitivity limits extrapolated for the scanning mode were found to be ~ 2x 10^{-12} M (S/N = 3) by flowing 1 x 10^{-11} M fluorescein through an open capillary. The sensitivity limits for the stationary mode were found to be ~ 1 x 10^{-12} M. These detection limits are at least as good as those reported from single capillaries using the conventional 90° detection geometry[10]. The background from the gel-filled capillaries was ~2.6 times (n = 4) higher than that from a capillary filled with just TBE buffer. Thus, the presence of the gel increased the background noise by a factor of ~1.6.

   This work indicates that the overall throughput performance of CAE can be very high. In the present study, satisfactory sequencing information is obtained out to 500 bases for each of four capillaries. The overall throughput of the system depends upon the total number of capillaries, N, that can be scanned. The equation, N - vT/2D, defines how N depends on the scan speed (v), the scan repetition period (T), and the capillary outside diameter (D). For example, one hundred 200 μm wide capillaries can be easily seen using a scan rate of four cm/sec and a one-sec scan repetition period. Increasing the array size would require (1) an increase in the scan speed; (2) the use of small r O.D. capillaries; and (3) an incr ase in the scan r p tition period which would reduce the temporal resolution of the

electrophoretic separation. Since reliable systems have velocities up to ten cm/sec and capillaries with O.D.'s of 150μm are commercially available, a limit of approximately 330 capillaries/array can be projected, assuming a ne-

5   second scan repetition period.

To illustrate our ability to extend this system to large numbers of capillaries we present, in Figure 8, an array of 24 capillaries that have been used to separate a different DNA sequencing sample.

10   Finally, it should be noted that there is a significant difference in the migration time of a given DNA band from lane-to-lane. This may be caused by inhomogeneities of the gel matrix or the presence of local non-uniform variations in the electric field strength. It has previously been

15   estimated that there is a 5% variation in migration time between identical samples on different gel columns[4].

The velocity shift of the DNA bands from lane-to-lane may preclude sequencing DNA with CAE using a single fluorophore and four different capillaries, one for each

20   base. For DNA sequencing, the present apparatus must be expanded to a multi-color detection system to sequence all four bases in a single capillary. Such four-color detection schemes have been developed for single capillaries[8] and for slab gels[19]. The basic idea is that one is separating four

25   sets of DNA fragments which terminate with either a G, A, T or C. Each set is labeled with a different fluorescent tag by any of several procedures and then the fragment sets are pooled and separated on the same capillary. If the fluorescent tags emit in a sufficiently distinctive

30   wavelength region, the four sets of fragments can be uniquely detected by using a four-color detection system.

A schematic diagram of a four-color confocal fluorescence capillary array scanner is shown in Figure 9. The scanner includes a laser source such as an argon laser

35   which projects a beam 30 into the dichroic beamsplitter 31 which directs the beam to the objective 32. The objective coll cts the fluorescent nergy from th focal volume and directs it through the beamsplitter. The output of the beam

-14-

splitter is direct d to a first beam splitter 33 which
reflects energy at on wavelength, for example, 540 μm, and
passes other, longer wavelengths. The next dichr ic
beamsplitter 34, which reflects energy at a second
5   wavelength, for example, 560 μm, and passes other, longer
wavelengths. A third beamsplitter 36 reflects energy at
another wavelength, for example, 580 μm, and passes energy
at 610μm. The energy from each of the beamsplitters 33, 34,
and 36 and the transmitted energy is applied to confocal,
10   spatial and spectrial filters 37, 38, 39 to photomultipliers
41, 42, 43 and 44 which provide output signals that are
processed and applied to computer 47 which generates an
image for each of the wavelengths, for each of the
capillaries. Each color image then records the passage of
15   a particular labeled set of DNA sequencing fragments through
the detection zone--one color for the A-fragments, a second
for the G-fragments, a third for the T-fragments and a
fourth for the C-fragments.

Throughout the preceding description and drawings,
20   reference has been made to capillary arrays. It should be
recognized that even though capillary arrays comprising a
plurality of capillary tubes have been shown and discussed
that it is possible to form parallel capillary passages in
a block of material by photoetching, micromachining, casting
25   and other techniques used in the semiconductor industry.
Thus, capillary array as used herein is meant to encompass
all types of capillary passages arranged in an array.

The preceding description is based on continuous
scanning across the capillary array. In some applications,
30   it may be desirable to focus sequentially at the center of
each capillary and step between capillaries. Finally, it is
possible to scan across each capillary to scan the band, but
then rapidly step or move to the next capillary.

In summary, it has been shown that it is possible to
35   perform high-sensitivity fluorescence detection of capillary
arrays using a confocal fluorescence scanner. This format
has the advantages that (1) many analytes can be run in
parallel; (2) loading multiple samples can be easily

accomplished; (3) rapid separations are achieved; and (4)
the detection sensitivity is excellent.   Use of capillary
arrays resolves the fundamental throughput problems that
limit the utility of CE in, for example, DNA sequ ncing[29].
5   In addition, CAE provides an opportunity for the large-scale
optimization of analytical separations. A large number of
capillaries can be run in parallel each with a different
buffer pH, buffer composition, or load to determine the best
separation conditions. Commercially made capillary arrays
10 could be constructed which plug into multi-well devices for
large-scale parallel sample introduction. CAE should be a
valuable new technique for rapid, parallel separation and
analysis. The apparatus has been described in connection
with capillary array electrophoresis. However, it is to be
15 understood that it can be used in connection with other
types   of   capillary   separations,   such   as   capillary
chromatography, isoelectric focusing and column derivations.
The foregoing descriptions of specific embodiments of
this invention have been presented for purposes of illus-
20 tration and description.   They are not intended to be
exhaustive or to limit the invention to the precise forms
disclosed, and many modifications and variations are pos-
sible in light of the above teaching. The embodiments were
chosen and described in order to best explain the principles
25 of the invention and its practical application, to thereby
enable others skilled in the art to best use the invention
and various embodiments with various modifications as are
suited to the particular use contemplated. It is intended
that the scope of the invention be defined by the claims
30 appended hereto and their equivalents.

-16-

1   Jorgenson, J.W.; Lukacs, K.D.; *Science* 1983, *222*, 266-272

2   Gordon, M.J.; Huang, X.; Pentoney, S.L., Jr.; Zare,R.N.; *Sci nce* 1988, *242*, 224-228

3   Ewing, A.G.; Wallingford, R.A.; Olefirowicz, T.M.; *Anal.Chem.* 1989, *61*, 292A-303A

4   Karger, B.L.; Cohen, A.S.; Guttman, A.; *J.Chromatogr.* 1989, *492*, 585-614

5   Kuhr, W.G.; *Anal.Chem.* 1990, *62*, 405R-414R

6   Cohen, A.S.; Najarian, D.R.; Paulus, A.; Guttman, A.; Smith, J.A.; Karger, B.L.; *Proc.Natl.Acad.Sci.USA*, 1988, *85*, 9660-9663

7   Heiger, D.N.; Cohen, A.S.; Karger, B.L.; *J. Chromatogr.* 1990, *516*, 33-48

8   Luckey, J.A.; Drossman, H.; Kostichka, A.J.; Mead, D.A.; D'Cunha, J.; Norris, T.B.; Smith, L.M.; *Nucleic Acids Res.* 1990, *18* 4417-4421

9   Swerdlow, H.; Wu, S.; Harke, H.; Dovichi, N.J.; *J. Chromatogr.* 1990, *516*, 61-67

10 Swerdlow, H.; Gesteland, R.; *Nucleic Acids Res.* 1990, *18*, 1415-1419

11 Drossman, H.; Luckey, J.A.; Kostichka, A.J.; D'Cunha, J.; Smith, L.M.; *Anal.Chem.* 1990, *62*, 900-903

12 Compton, S.W.; Brownlee, R.G.; *Bio Techniques* 1988, *6*, 432-439

13 Cohen, A.S.; Najarian, D.; Smith, J.A.; Karger, B.L.; *J. Chromatogr.* 1988, *458*, 323-333

14 Cheng, Y.F.; Dovichi, N.J.; *Science* 1988, *242* 562-564

15 Hjerten, S.; Elenbring, K.; Kilar, F.; Liao, J.L.; Chen, A.J.C.; Sieberg, C.J.; Zhu, M.D.; *J. Chromatogr.* 1987, *403*, 47-61

16 Gassmann, E.; Kuo, J.E.; Zare, R.N.; *Science* 1985, *230*, 813-814

17 Ansorge, W.; Sproat, B.; Stegemann, J.; Schwager, C.; Zenke, M.; *Nucleic Acids Res.* 1987, *15*, 4593-4602

18 Brumbaugh, J.A.; Middendorf, L.R.; Grone, D.L.; Ruth, J.L.; *Proc.Natl.Acad.Sci. USA* 1988, *85*, 5610-5614

19　Smith, L.M.; Sand rs, J.Z.; Kaiser, R.J.; Hughes, P.;
Dodd, C.; Connell, C.R.; Heiner, C.; Kent, S.B.H.;
Hood, L.E.; *Nature* 1986, *321*, 674-679

20　Zagursky, R. J.; McCormick, R. M.; *Bio Techniques* 1990,
9, 74-79

21　Prober, J.M.; Trainor, G.L.; Dam, R.J.; Hobbs, F.W.;
Robertson, C.W.; Zagursky, R.J.; Cocuzza, A.J.; Jensen,
M.A.; Baumeister, K.; *Science* 1987, *238*, 336-341

22　Glazer, A.N.; Peck, K.; Mathies, R.A.;
Proc.Natl.Acad.Sci. USA 1990, *87*, 3851-3855

23　Mathies, R.A.; Peck, K.; Stryer, L.; *Bioimaging and
Two-Dimensional Spectroscopy*, L.C. Smith (ed.), SPIE-
The International Society for Optical Engineering,
Bellingham, WA. 1990, 52-59

24　Rye, J.S.; Quesada, M.A.; Peck, K.; Mathies, R.A.;
Glazer, A.N.; *Nucleic Acids Res.* 1991, *19*, 327-333

25　Quesada, M.A.; Rye, H.S.; Gingrich, J.C.; Glazer,
A.N.; Mathies, R.A.; *Bio Techniques* 1991, *10* 616-625

26　L. Hernandez, R. Marquina, J. Escalona, N.A. Guzman, *J.
Chromatogr.* 502, 247-255 (1990).

27　L. Hernandez, J. Escalona, N. Joshi, N. Guzman, *J.
Chromatogr.* 559, 183-196

28　Smith, L.M.; *Nature* 1991, *349*, 812-813

WHAT IS CLAIMED:

1.   A scanner for xciting and detecting radiation from a plurality f adjacent capillary passages comprising

a plurality of side-by-side capillary passages disposed in a plane,

a source of radiant energy of a first wavelength,

an objective lens for receiving and focusing said radiant energy at an excitation volume in the plane of said passages,

means for moving said passages so that said excitation volume sequentially and repetitively is within one of said capillary passages to excite material in said passage and cause the material to radiate energy at a different wavelength,

said objective lens serving to collect said radiant energy and direct it to an optical system which includes confocal spatial and spectral filter means to transmit emitted radiant energy at said different wavelength and reject radiation at other wavelengths,

a detection system for receiving said emitted radiation and generate a signal, and

computer means for receiving and processing said signal to provide an output representative of the material at the excitation volume in each of said capillary passages.

2.   A scanner as in Claim 1 in which said means for moving said passages is controlled by said computer, whereby the output can be correlated with the capillary passages.

3.   A scanner as in Claim 1 in which said means for moving the passages moves continuously whereby to scan a band in each of said capillaries.

4.   A scanner as in Claim 1 in which said means for moving said passages steps the passages into said excitation v lume.

5.   A scanner as in Claim 2 in which th capillary passages are part of an elongated cylindrical capillary.

6.   A scanner as in Claim 5 including means for holding a region of said capillaries in side-by-side co-planar relationship for presentation to the focused radiant energy.

7.   A scanner as in Claim 6 in which the ends of said capillaries are separable for individual manipulation and loading.

8.   A scanner as in Claim 1 wherein said excitation radiant energy excites material which emits radiant energy at a plurality of wavelengths and said optical system includes a plurality of confocal, spatial and spectral filter means for selectively directing the radiant emitted energy of different wavelengths to different locations and a plurality of detection means each receiving emitted energy at a selected wavelength and providing a corresponding output signal.

9.   An apparatus for exciting and detecting radiation from sample material in capillary passages comprising
         means for presenting a plurality of capillary passages in side-by-side coplanar relationship,
         radiation means for exciting sample material in said passages with radiation of a first wavelength;
         means for moving said plurality of side-by-side coplanar capillary passages to sequentially and repetitively excite sample material in each passage with said radiation,
         means for collecting radiation emitted from said sample,
         means for detecting radiation emitted by the sample in said passages responsive to said excitation radiation, and
         computer means for processing the detected emitted radiati n and for controlling movem nt of said moving means to providing a two-dimensional output repr s ntative of the

sample in said capillary passages as a function of time and
position.

10. A scanner as in Claim 9 in which said capillary
passages are part of an elongated cylindrical capillary.

11. A scanner as in Claim 10 including means for holding a
region of said capillaries side-by-side coplanar
relationship for presentation to the focused radiant energy.

12. A scanner as in Claim 9 in which the ends of said
capillaries are independently manipulatable.

13. A scanner for detection of fluorescently labeled
analytes which can be separated in small diameter
capillaries comprising
     a plurality of capillaries each for separating a
fluorescently labeled analyte
     a source of radiant energy having a wavelength which
excites fluorescence from said labeled analyte,
     lens means for focusing said radiant energy to a small
volume and for collecting emitted energy from said volume
     means for sequentially and repetitively presenting a
region of said plurality of capillaries to said volume of
radiant energy whereby to cause fluorescence of the
fluorescently labeled analyte at said volume, and;
     means for receiving the collected fluorescent radiation
and providing an output representative of the analyte at
said volume.

14. A scanner as in Claim 13 in which said lens means
comprises an objective lens forming a part of a confocal
optical detection assembly including a spatial filter.

15. A scanner as in Claim 14 in which said optical
detection system includes sp ctral filters for rejecting
energy at said excitation wavelength.

16. A method of detecting fluorescence from DNA sequencing fragm nts electrophoretically separated in a plurality of capillaries which comprises

positioning a region of said plurality of capillaries in side-by-side coplanar relationship

exciting a predetermined volume sequentially and repetitively in said capillaries with light energy of predetermined wavelength focused therein by an objective lens to cause fragments to fluoresce at a different wavelength,

collecting the fluorescently emitted light from said predetermined volumes in each of said capillaries with said objective lens,

spectrally and spatially filtering said fluorescently emitted light energy of different wavelengths to reject light at said predetermined wavelengths and passing said emitted light; and,

applying the filtered emitted light to a detector to generate an output signal representative of the fluorescence from said fragments in each of said capillaries.

17. The method of Claim 16 in which one end of the capillaries is separated for rapid parallel loading of sequencing fragments into the end of the capillaries.

18. A scanner for exciting and detecting radiation from a plurality of adjacent capillary passages comprising

a plurality of side-by-side capillary passages disposed in a plane,

a source of radiant energy of a first wavelength,

an objective lens for receiving and focusing said radiant energy at an excitation volume in the plane of said passages,

a beamsplitter for directing said radiant energy to the objective lens to excite said excitation volume with energy at said wavelength,

means for moving said passages so that said excitation volum sequentially and repetitively is within ne of said

capillary passages to excit material in said passage and
cause the material to radiate energy at a different
wavelength,

said objective lens serving to collect said radiant
energy and direct it through said beamsplitter which passes
radiated energy at said different wavelength to an optical
system which includes a confocal spatial filter and spectral
filters to transmit emitted radiant energy at said different
wavelength and reject radiation at other wavelengths,

a detection system for receiving said emitted radiation
and generate a signal, and

computer means for receiving and processing said signal
to provide an output representative of the material at the
excitation volume in each of said capillary passages.

19. A scanner as in Claim 18 in which said means for moving
said passages is controlled by said computer, whereby the
output can be correlated with the capillary passages.

20. A scanner as in Claim 19 in which said means for moving
the passages moves continuously whereby to scan a band in
each of said capillaries.

21. A scanner as in Claim 18 in which said means for moving
said passages steps the passages into said excitation
volume.

22. A scanner as in Claim 19 in which the capillary
passages are part of an elongated cylindrical capillary.

23. A scanner as in Claim 22 including means for holding a
region of said capillaries in side-by-side co-planar
relationship for presentation to the focused radiant energy.

24. A scanner as in Claim 23 in which the ends of said
capillaries are separable for individual manipulation and
loading.

25. A scanner as in Claim 18 wherein said excitation radiant energy excites mat rial which emits radiant energy at a plurality of wavelengths and said optical system includes a plurality of additional beamsplitters, confocal, spatial and spectral filter means for selectively directing the radiant emitted energy of different wavelengths to different locations and a plurality of detection means each receiving emitted energy at a selected wavelength and providing a corresponding output signal.

26. A method of detecting fluorescence from DNA sequencing fragments electrophoretically separated in a plurality of capillaries which comprises

positioning a region of said plurality of capillaries in side-by-side coplanar relationship

exciting a predetermined volume sequentially and repetitively in said capillaries with light energy of predetermined wavelength focused therein by an objective lens to cause fragments to fluoresce at a different wavelength,

collecting the fluorescently emitted light from said predetermined volumes in each of said capillaries with said objective lens,

spectrally and confocally spatially filtering said fluorescently emitted light energy of different wavelength to reject light at said predetermined wavelengths and scattered light, and passing said emitted light; and,

applying the filtered emitted light to a detector to generate an output signal representative of the fluorescence from said fragments in each of said capillaries.

27. The method of Claim 26 in which one end of the capillaries is separated for rapid parallel loading of sequencing fragments into the end of the capillaries.

28. The meth d of Claim 26 in which said fragments flu resc at a plurality of wavelengths and spectrally and confocally spatially filtering said fluorescently emitted
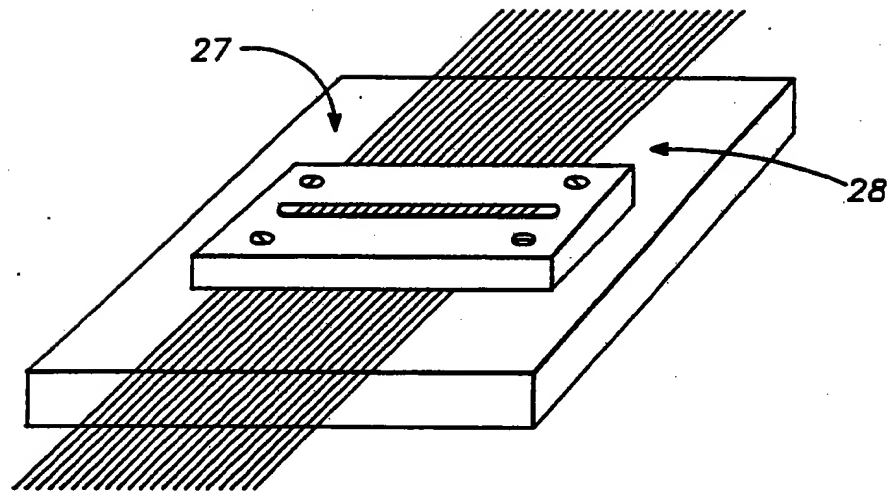
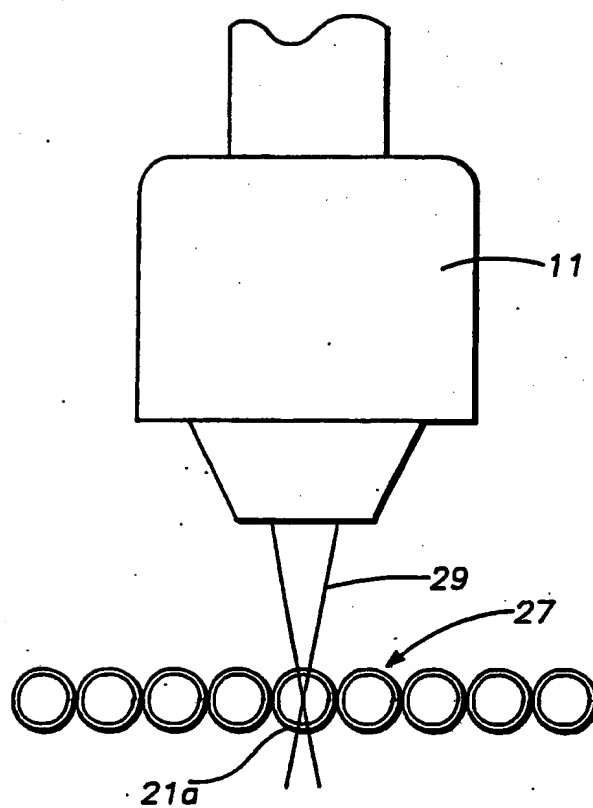light energy at each of said plurality of wavelengths and applying it to corresponding detectors.

COMPUTER

PREAMPLIFIER

STAGE-CONTROLLER

HIGH VOLTAGE
POWER SUPPLY

18

17

16

14

12

13

23

26

28

27

11

30

21

22

24

FIG. -1

27

28
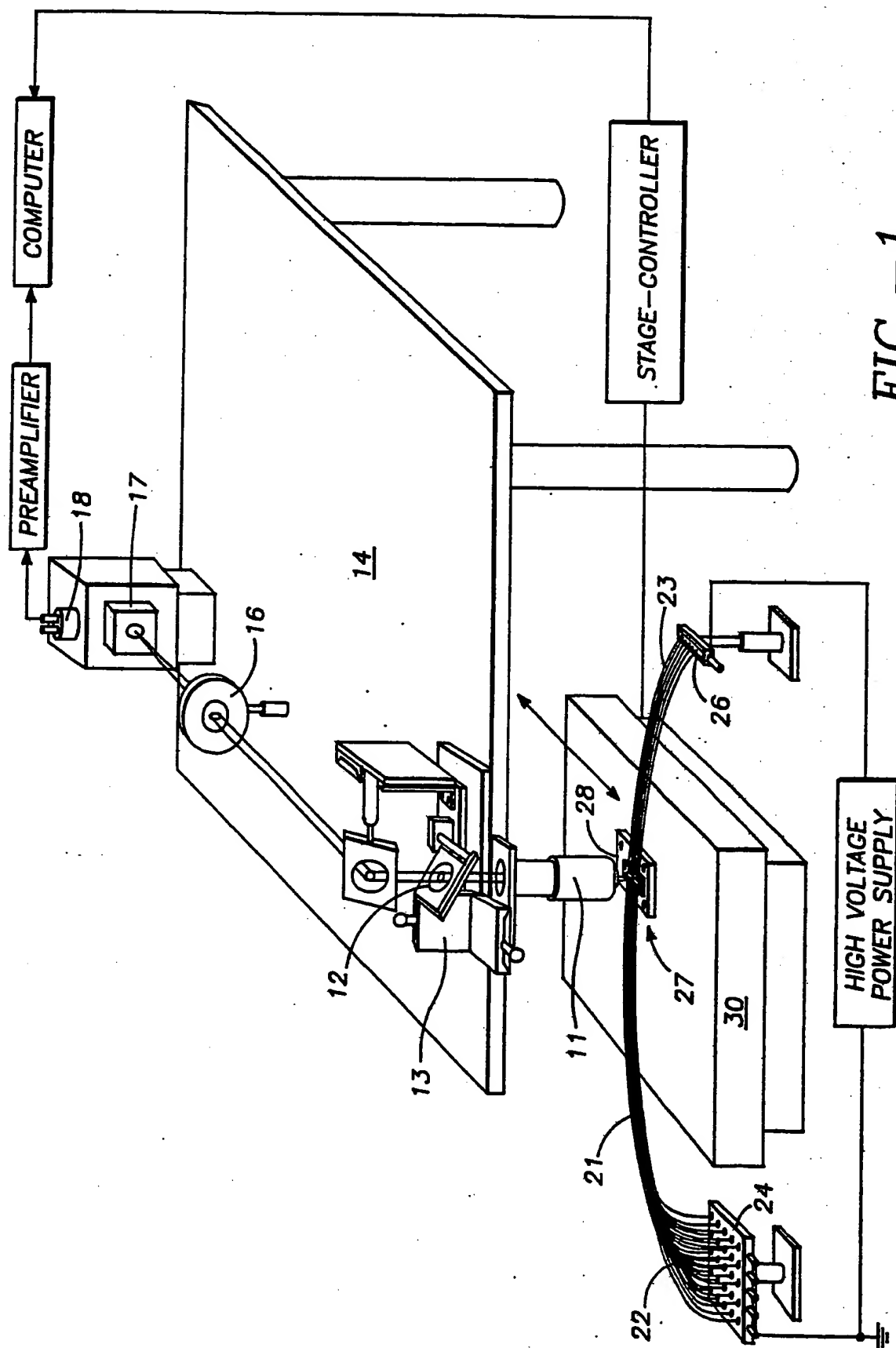
FIG.—2

FIG. -3
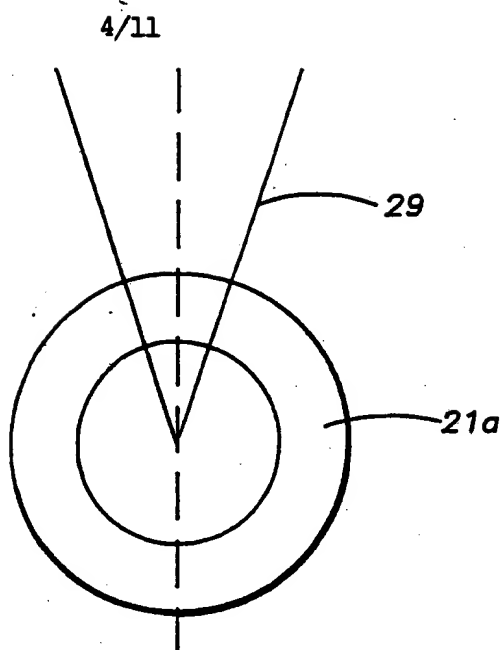
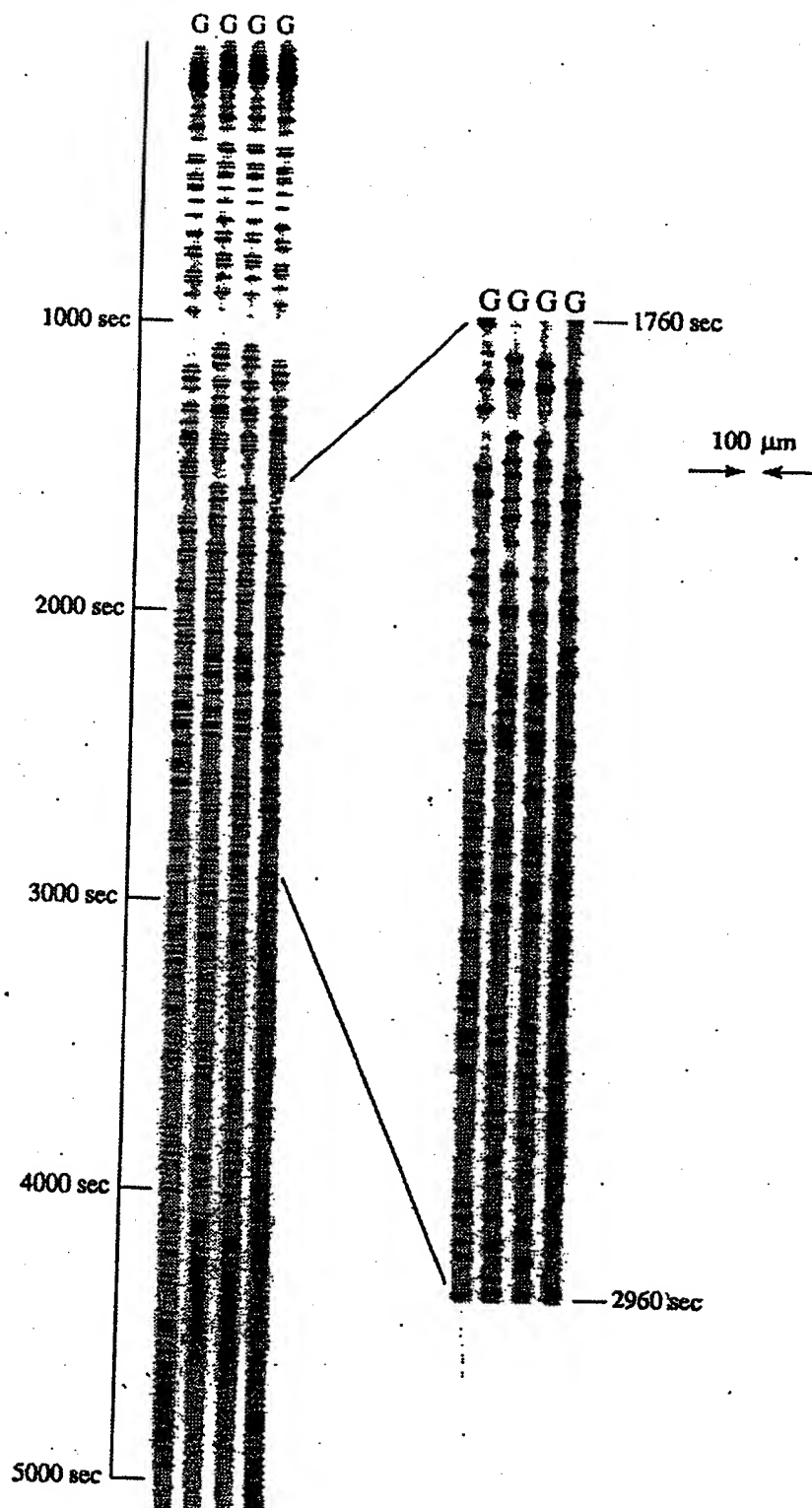*FIG.—4A*
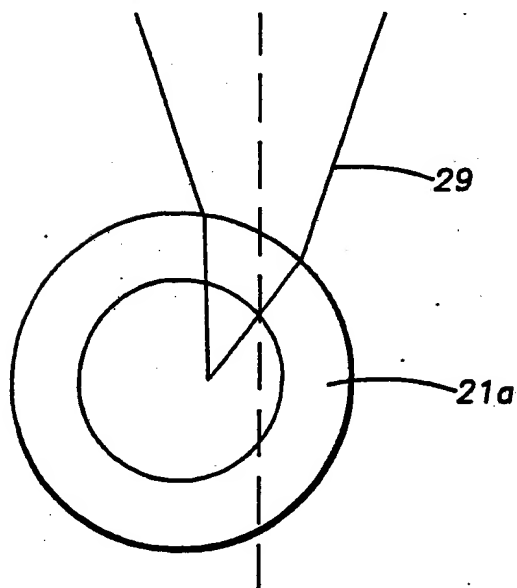
FIG.—4B

FIG.—5
SUBSTITUTE SHEET
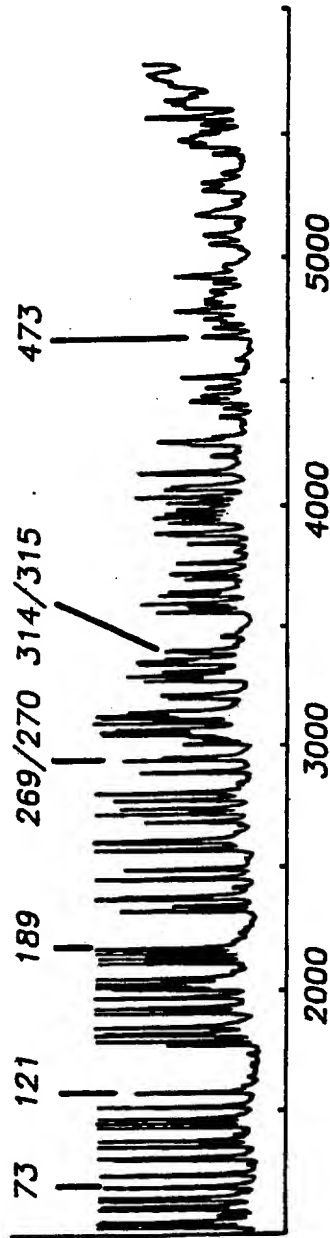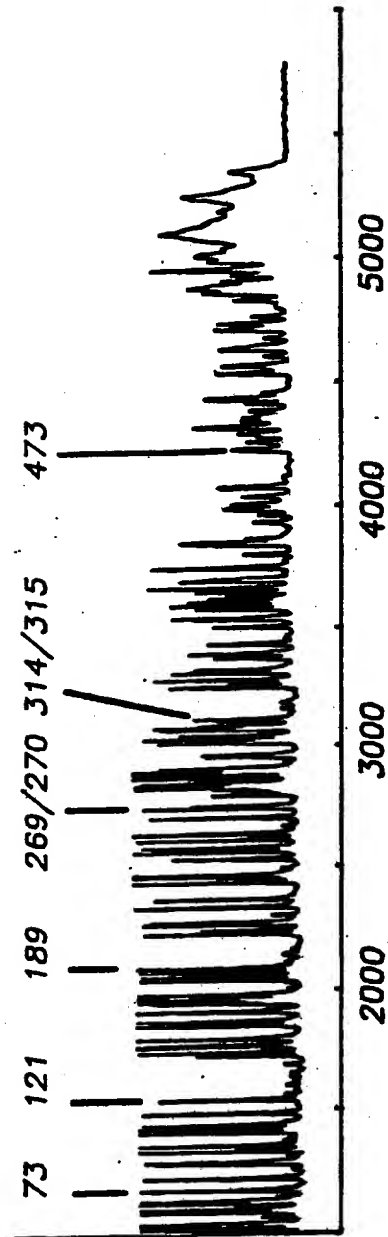
FIG. -6A
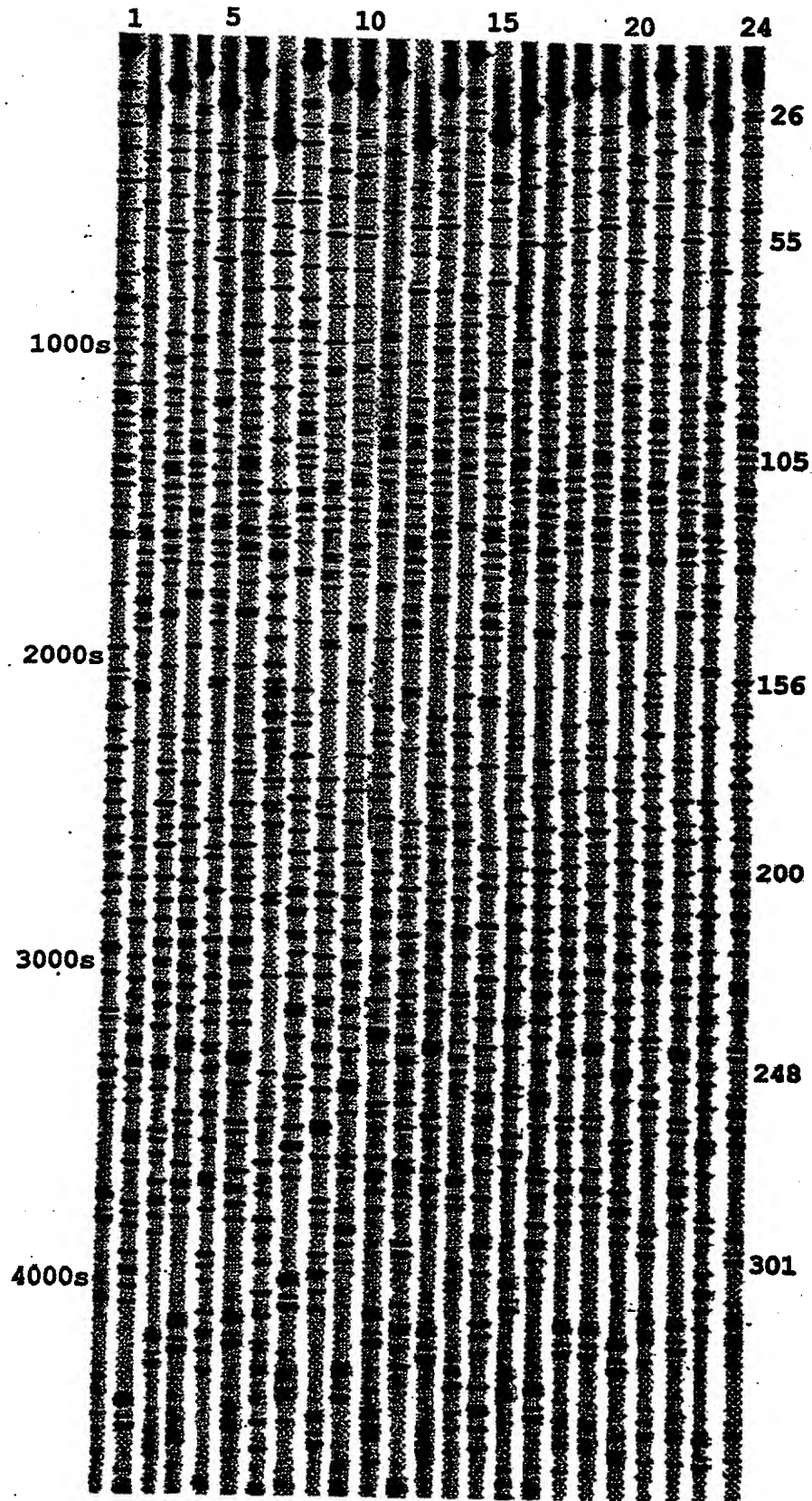

FIG. -6B
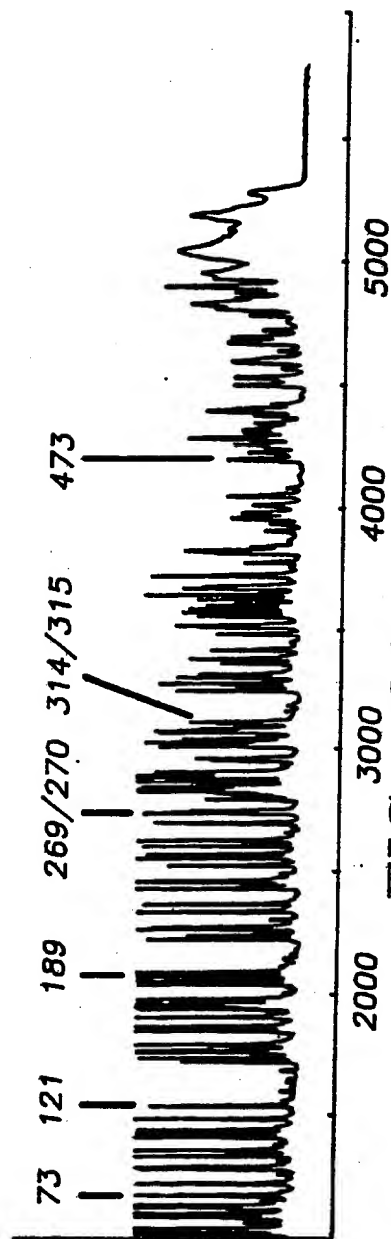
FIG.—6C
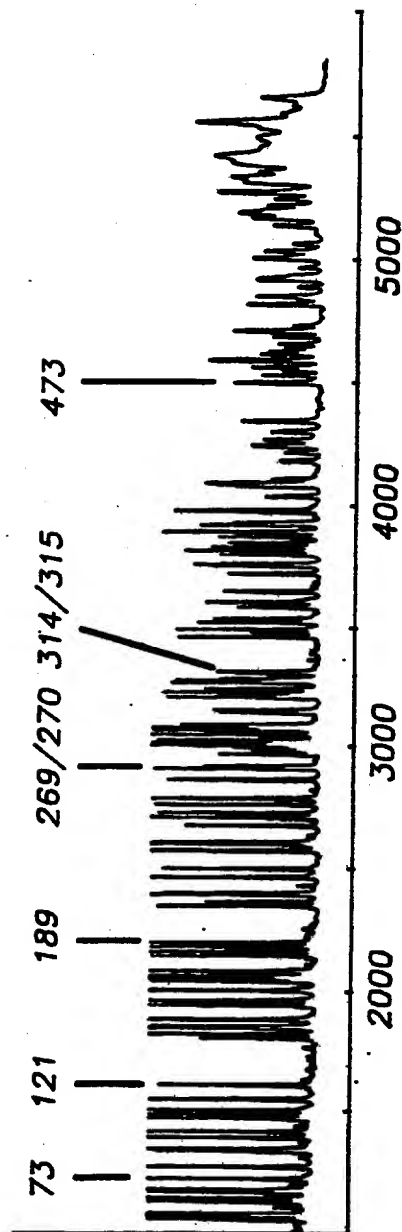
FIG.—6D
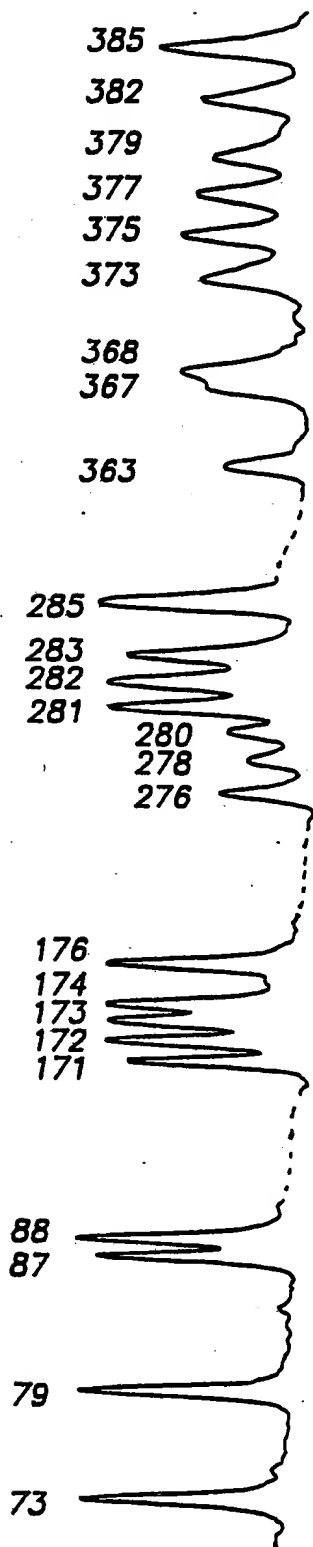
FIG. — 7A
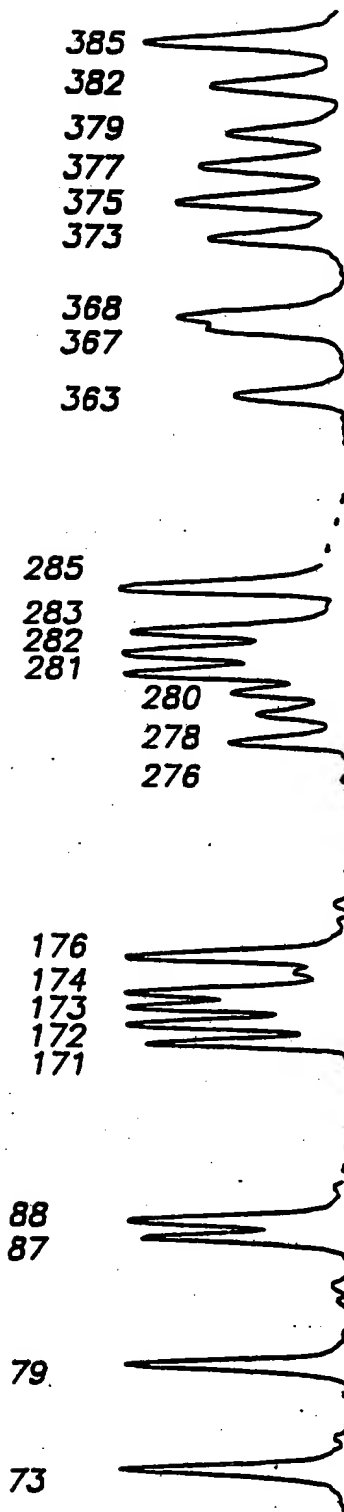
FIG. — 7B
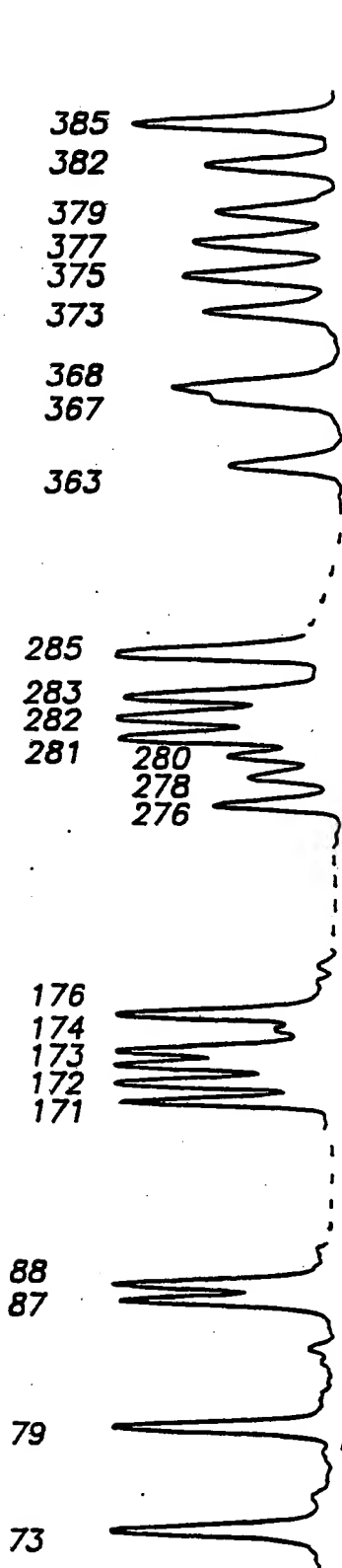
FIG. – 7C
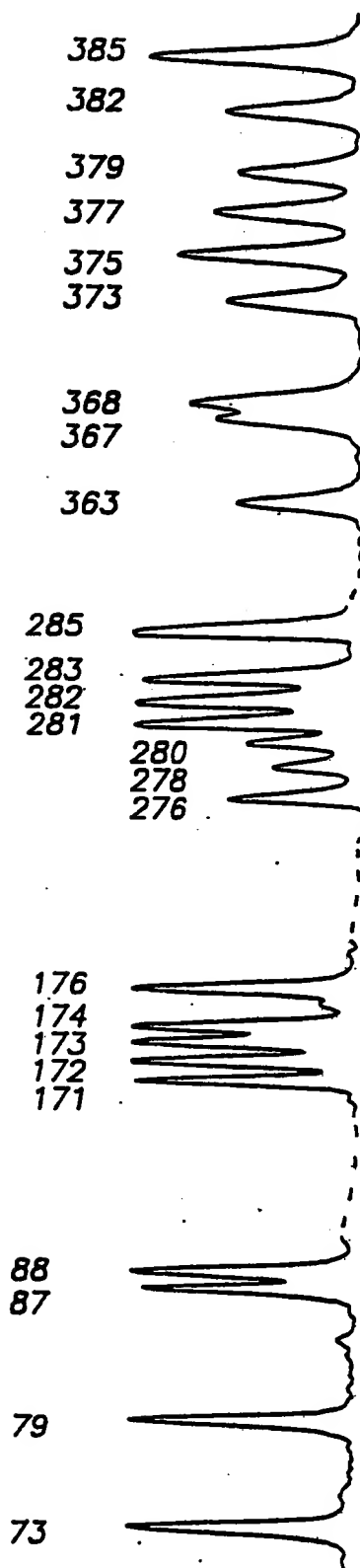
FIG. – 7D

FIG. –8

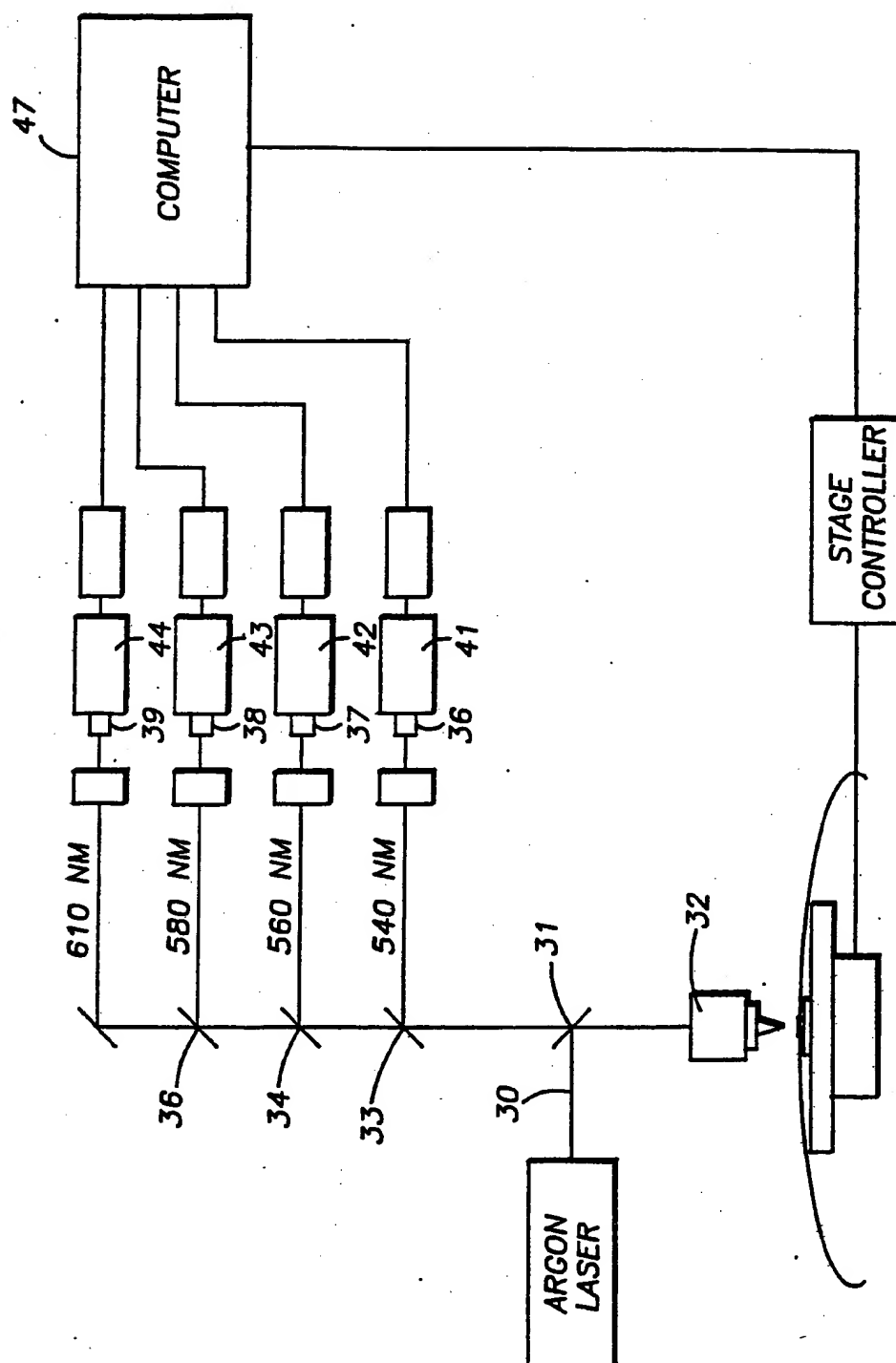FIG. –9

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :G01N 21/64
US CL :250/458.1, 459.1, 461.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 250/458.1, 459.1, 461.1 356/344; 204/180.1,182.1,299R,182.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Automated Patent System (APS): Capillary + Electrophoresis; Capillar? (ZA) (Array or Plurality)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US,A, 5,091,652 (Mathies et al.) 25 February 1992 See entire document.	1-28
Y	SPIE-Bioimaging and Two-Dimensional Spectroscopy, Vol. 1205, 1990, Mathies et al., "High-Sensitivity Single-Molecule Fluorescence Detection", pp. 52-59 See entire document.	1-7,9-24 26 and 27
Y	Biotechniques, Vol. 9, No. 1, 1990 Zagursky et al., "DNA Sequencing Separation in Capillary Gels on a Modified Commercial DNA Sequencing Instrument", pp. 74-78 See entire document.	1-7,9-24 26 and 27

[X] Further documents are listed in the continuation of Box C.   [ ] See patent family annex.

*   Special categories of cited documents:

"A"   document defining the general state of the art which is not considered to be part of particular relevance

"E"   earlier document published on or after the international filing date

"L"   document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O"   document referring to an oral disclosure, use, exhibition or other means

"P"   document published prior to the international filing date but later than the priority date claimed

"T"   later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"   document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"   document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"   document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 APRIL 1993	11 MAY 1993

Name and mailing address of the ISA/US	Authorized Officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	EDWARD J. GLICK
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-4858

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
Y	Nucleic Acids Research, Vol. 18, No. 15, 1990, Luckey et al., "High-Speed DNA Sequencing by Capillary Electrophoresis", pp. 4417-4421 See entire document.	8,25 and 28